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ENERGY METABOLISM DURING AIR EXPOSURE AND RECOVERY IN THE HIGH INTERTIDAL BIVALVE MOLLUSC *GEUKENSIA DEMISSA GRANOSISSIMA* AND THE SUBTIDAL BIVALVE MOLLUSC *MODIOLUS SQUAMOSUS*

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Abstract

Metabolic responses to air exposure and recovery were investigated in the adductor muscles of the high intertidal mussel *Geukensia demissa granosissima* and the subtidal mussel *Modiolus squamosus*. Exposure to air for 12 h had no significant effect on the levels of high energy phosphates (arginine phosphate, ATP) in the adductor muscles of *G. demissa granosissima,* indicating minimal metabolic stress in this species. In contrast, there was a considerable decline in arginine phosphate and ATP during air exposure in the phasic and tonic adductor muscles of *M. squamosus*. In addition, there was a substantial accumulation of alanine and succinate under these conditions. Furthermore, D-lactate accumulated in the phasic muscle of *M. squamosus* during air exposure. During recovery, there were transient accumulations of alanopine/strombine in both *G. demissa granosissima* and *M. squamosus*. The differences in metabolic responses between these two species reflect adaptations to specific microhabitats. It appears that metabolism in the posterior adductor muscle of *G. demissa granosissima* is largely aerobic during air exposure. The subtidal species *M. squamosus* displays a much greater reliance on anaerobic pathways of energy production under these conditions.

Introduction

Bivalve molluscs are not structurally well adapted for aerial gas exchange (Lent, 1968). The gills show extensive modifications for filter feeding and, secondarily, for gas exchange. The role of the gills in gas exchange may be quite reduced in some species. Booth and Mangum (1978) showed that ligation of the aorta of the ribbed mussel *Modiolus demissus (Geukensia demissa)* resulted in only a 15% decrease in aquatic oxygen consumption. Thus, gas exchange in this species may take place primarily over the generalized body surfaces. During exposure to air at low tides, many marine bivalves appear to be capable of taking up atmospheric oxygen. Significant rates of aerial oxygen consumption have been observed in *Cerastoderma edule* (Boyden, 1972), *Mytilus edulis* (Coleman, 1973), *Modiolus modiolus* (Coleman, 1976), and *Modiolus demissus* (Booth and Mangum, 1978). Typically, rates of aerial gas exchange are lower than aquatic rates (Coleman, 1973; Bayne *et al.,* 1976; Widdows *et al.,* 1979).

The metabolic rates of bivalve molluscs exposed to air vary considerably between species and, in a temporal sense, may vary considerably within an individual. For

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instance, Pamatmat (1983) measured heat production rates during air exposure in specimens of *Geukensia demissa*. Animals tended to show regular cycles of high rates of heat production (valves presumably open, metabolism principally aerobic) followed by low rates of heat production (valves presumably closed, metabolism principally anaerobic). The period of the cycle varied from individual to individual (Pamatmat, 1983). The relative contributions of anaerobic energy yielding processes to the total metabolic rate may depend on the previous acclimation history of the individual. Shick and Widdows (1981) showed, using calorimetric techniques, that subtidally acclimated specimens of *M. edulis* relied exclusively on anaerobic metabolism during air exposure. Experiments with subtidally acclimated specimens of the cockle *Cardium edule* indicated that metabolism was exclusively aerobic during air exposure. In contrast, anaerobic heat production accounted for 62% of the total heat production in intertidally acclimated specimens of *M. edulis* exposed to air (Shick and Widdows, 1981).

Anaerobic metabolism has been studied extensively in bivalve molluscs (de Zwaan, 1977). There are a variety of metabolic options available for energy production during air exposure and anoxia. Lactate production is not common, although it is a major end product in at least one bivalve mollusc (Gäde, 1980). Typically, there is a simultaneous fermentation of glycogen and aspartate yielding succinate and alanine as end products (Collicutt and Hochachka, 1977; Ebberink *et al.*, 1979). Aspartate provides the carbon skeleton for succinate and the amino group used in alanine formation. Assuming that once aspartate levels become depleted, further alanine formation is minimal and succinate carbon is then derived exclusively from glycogen. This metabolic transition may involve a shift at the phosphoenolpyruvate (PEP) branchpoint involving increased activity of the enzyme phosphoenolpyruvate carboxykinase (PEPCK) (Ebberink *et al.*, 1979). Recently, de Zwaan *et al.* (1982) questioned the role of PEPCK in the energy metabolism of the posterior adductor muscle of *M. edulis*. During extended anoxia, the volatile fatty acid, propionate, has also been shown to be a major end product in specimens of *M. edulis* (Kluytmans *et al.*, 1975, 1978).

In addition to lactate, alanine, succinate, and propionate, an entirely new class of end products has recently been shown to accumulate during anoxia. Fields (1976) discovered a cytoplasmic dehydrogenase in oyster tissues which utilized pyruvate and an amino acid as substrates. The resulting products of the reaction were the iminodicarboxylic acids, alanopine (alanine as substrate), and strombine (glycine as substrate). Recently, it has been shown that strombine accumulates during air exposure in the posterior adductor muscles of specimens of *M. edulis* (Zurburg *et al.*, 1982; de Zwaan *et al.*, 1983).

Regardless of the qualitative nature of the end products produced during air exposure, bivalve molluscs display great similarities with respect to the overall magnitude of energy metabolism. A Pasteur effect is typically absent (de Zwaan, 1977). Thus, there is no increase in glycolytic flux during anoxia and consequentially, rates of ATP production fall. De Zwaan and Wijsman (1976) and Ebberink *et al.* (1979) showed that the energy expenditure of the adductor muscle of *M. edulis* decreases on the order of five fold during air exposure. The diminished energy demand tends to maintain energy balance despite low rates of glycolytic flux.

Investigation into the metabolic events immediately following oxygen stress has lagged far behind studies dealing with metabolism during air exposure. A variety of metabolic events occur during recovery including recharging of high energy phosphates, oxidation of end products, and resynthesis of anaerobic substrates. Typically, levels of succinate, lactate, and alanine fall while aspartate levels rise (Gäde and Meinardus,

1981; Zurburg *et al.,* 1982). The resynthesis of ATP and the phosphagen, arginine phosphate, also occurs during recovery. Most molluscs show a characteristic elevation of oxygen consumption or oxygen debt following hypoxia reflecting, to some extent, the enhanced energy demand of recovery (de Zwaan, 1977; de Vooys and de Zwaan, 1978). In addition, there may be enhanced glycolytic flux, as strombine accumulates during recovery in at least one species (Zurburg *et al.,* 1982; de Zwaan *et al.,* 1983).

In the present study, we compare metabolic responses to air exposure and recovery in two species of bivalve molluscs adapted to distinctly different micro-habitats. The ribbed mussel *Geukensia demissa granosissima* is a high intertidal species which is regularly exposed to air for hours or even days at a time. The mussel *Modiolus squamosus* is a subtidal species. Populations of *M. squamosus* are exposed to air only during exceptionally low tides. The present study shows dramatic differences in terms of the metabolic responses of the two species to experimental air exposure. Specimens of *G. demissa granosissima* appear to rely extensively on aerial gas exchange showing only trivial accumulations of anaerobic end products. In contrast, specimens of *M. squamosus* show substantial accumulations of anaerobic end products indicating a reliance on anaerobic energy production during air exposure.

Materials and Methods

Animals

Specimens of *Geukensia demissa granosissima* were collected in salt marshes at Yent Bayou, Florida. Specimens of *Modiolus squamosus* were collected off Alligator Point, Florida. Animals were maintained in running sea water (24–28°C, 30‰) at the Florida State University Marine Laboratory, Turkey Point. Animals were used in experiments four to seven days after collection.

Materials

Biochemicals were purchased from Sigma Chemical Company (St. Louis, Missouri) and Boehringer Mannheim (Indianapolis, Indiana). All other chemicals were reagent grade quality. Octopine dehydrogenase, alanopine dehydrogenase, and D-lactate dehydrogenase were purified by affinity chromatography from the adductor muscle of the scallop *Argopecten irradians concentricus,* the adductor muscle of the oyster *Crassostrea virginica,* and muscle of the horseshoe crab *Limulus polyphemus.* These enzymes were used to assay for octopine, alanopine/strombine, and D-lactate, respectively. Succinyl Co A synthase, used in succinate assays, was a gift of Dr. William Bridger, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

Profile of adductor muscle enzyme activities

Activities of key glycolytic enzymes and citrate synthase were assayed in crude, cell-free extracts of the posterior adductor muscle of *G. demissa granosissima* and the phasic and tonic portions of the posterior adductor muscles of *M. squamosus.* The following enzymes were assayed: phosphorylase (Plase), hexokinase (HK), phosphofructokinase (PFK), lactate dehydrogenase (LDH), alanopine dehydrogenase (ADH), octopine dehydrogenase (ODH), glyceraldehyde-3-phosphate dehydrogenase (G-3PDH), and citrate synthase (CS). Tissue was homogenized in nine volumes (w:v) of extraction medium using a Brinkman Polytron tissue grinder and centrifuged at

10,000 × *g* for 20 min. The following extraction media were used: 50 m*M* triethanolamine containing 1 m*M* EDTA, 1 m*M* $MgCl_2$, and 30 m*M* 2-mercaptoethanol at pH 7.4 for LDH, ODH, ADH, PK, PEPCK, and HK; 70 m*M* Tris/HCl containing 1 m*M* EDTA and 5 m*M* $MgSO_4$ at pH 8.2 for PFK; 100 m*M* triethanolamine containing 7 m*M* 2-mercaptoethanol at pH 7.0 for Plase; and 25 m*M* Tris/HCl containing 1 m*M* EDTA at pH 7.5 for CS. Enzymes were assayed by standard procedures—Plase and G-3-PDH (de Zwaan *et al.*, 1980), PFK, HK, and PEPCK (Zammit and Newsholme, 1976), PK, LDH, ODH, and ADH (Ellington, 1981), and CS (Sugden and Newsholme, 1975). All assays were conducted in a Gilford 252-1 spectrophotometer at 25°C. Assays were initiated by the addition of substrate.

Metabolic responses to air exposure and recovery

Specimens of *G. demissa granosissima* and *M. squamosus* were collected and maintained in running sea water for four days prior to experimentation. At zero time, all animals were removed from the sea table and placed in a humidified (100%), temperature controlled (27°C) chamber. A total of 130 specimens of *G. demissa granosissima* and 144 specimens of *M. squamosus* were used in these experiments. A zero time group of animals (n = 10 for *G. demissa granosissima*, n = 12 for *M. squamosus*) was randomly selected, and the posterior adductor muscles were excised and frozen in liquid nitrogen. Phasic and tonic portions of the adductor muscle in *M. squamosus* were frozen separately. At various time intervals during air exposure (0.5 1, 2, 4, 7, and 12 h for *G. demissa granosissima;* 0.5, 1, 4, 7, and 12 h for *M. squamosus*) subsets of either 10 animals (*G. demissa granosissima*) or 12 animals (*M. squamosus*) were removed and posterior adductor muscles frozen. At the end of 12 h of air exposure, the remaining animals were returned to the sea table and subsets of animals were removed at various time intervals (2, 4, 6, 8, 10, and 12 h) and treated as above. All tissues were stored at −80°C. Tissues were processed and analyzed within 36 h of tissue sampling.

Biochemical analyses of tissue samples

Tissue samples were fragmented using a mortar and pestle chilled in liquid nitrogen. For each analysis, approximately 1 g of tissue representing the adductor muscles of several animals was weighed and homogenized in 5 volumes (w:v) 6% perchloric acid (4°C). The homogenates were centrifuged at 10,000 × *g* for 20 min and the supernatants neutralized with 5 *M* KOH/0.1 *M* $KHCO_3$. The neutralized extract was centrifuged and the supernatant stored at −80°C.

Arginine phosphate and ATP levels in the extracts were assayed within 3–5 h of extract preparation. Arginine phosphate and ATP were assayed by the spectrophotometric assays of Lowry and Passonneau (1972) except that lobster arginine phosphokinase was substituted for creatine phosphokinase. ADP and AMP were assayed according to Lowry and Passonneau (1972). Succinate was determined by the method of Williamson (1974). Octopine, alanopine/strombine and D-lactate were assayed in a reaction system consisting of 100 m*M* 2-amino-2-methyl-1-propanol (pH 9.2) containing 50 m*M* hydrazine, 4 m*M* NAD, and 10 m*M* EDTA. Assays were initiated by the addition of 5 enzyme units of the appropriate enzyme. Alanine, glycine, aspartate, and glutamate were determined using a Beckman model 120-1 automatic amino acid analyzer. Propionate levels were determined by HPLC. One (1) ml of the neutralized, perchloric acid extract was applied to a silica Sep-Pak (Waters, Inc.) pretreated with 1.0 ml ultra pure water followed by a 4.0 ml ultra pure hexane wash. The sample was then washed with 2.0 ml of ultra pure hexane and the polar fraction

eluted with 1.0 ml of ultra pure water. Treated extracts were analyzed on a Waters HPLC system using a BIO-RAD (Bio-Rad Laboratories, Richmond, California) ODS-5 reversed phase column (250 mm × 4 mm, ID), isocratic elution (0.2 M KH_2PO_4, pH 2.4), and UV detection (200 nm).

All metabolite data were analyzed for significance by one way ANOVA and a least significant difference test (Freyer, 1966).

Results

Profile of the activities of key glycolytic enzymes and citrate synthase

Activities of key glycolytic enzymes and citrate synthase in the adductor muscle of *G. demissa granosissima* and the phasic and tonic portions of the adductor muscle of *M. squamosus* are listed in Table I. In general, enzyme activities were similar when comparing the two species. However, ADH activity in both posterior adductor muscles of *M. squamosus* was one order of magnitude greater than activity in *G. demissa granosissima.* In addition, ODH was absent in the adductor muscles of *M. squamosus.* The adductor muscles of both species had relatively low phosphorylase and hexokinase

Table I

Activities of key glycolytic enzymes and citrate synthase in the posterior adductor muscles of G. demissa granosissima *and* M. squamosus

Enzyme		Enzyme activity[1]	
		M. squamosus	*G. demissa*
Lactate dehydrogenase	MP	1.47 ± 0.37	3.07 ± 0.38
	MT	0.84 ± 0.22	
Octopine dehydrogenase	MP	n/a	4.66 ± 1.24
	MT	n/a	
Alanopine dehydrogenase	MP	13.56 ± 2.04	1.02 ± 0.04
	MT	12.06 ± 2.76	
Pyruvate kinase	MP	2.49 ± 0.16	1.07 ± 0.01
	MT	2.26 ± 0.11	
Phosphoenolpyruvate carboxykinase	MP	3.11 ± 0.31	4.41 ± 0.03
	MT	2.53 ± 0.15	
Hexokinase	MP	0.02 ± 0.00	0.30 ± 0.03
	MT	0.02 ± 0.01	
Citrate synthase	MP	1.16 ± 0.08	2.34 ± 0.20
	MT	0.96 ± 0.09	
Glyceraldehyde-3-phosphate dehydrogenase	MP	42.75 ± 5.41	41.05 ± 3.42
	MT	26.15 ± 3.12	
Phosphorylase	MP	0.87 ± 0.02	1.24 ± 0.18
	MT	1.38 ± 0.15	
Phosphofructokinase	MP	4.27 ± 0.37	4.56 ± 0.75
	MT	3.32 ± 0.14	

[1] Enzyme activities are expressed as μmoles/(min · g wet wgt) at 25°C.

Each value represents a mean ± 1 S.D. (n = 4). MP = phasic adductor, Mt = tonic adductor, N/a = no activity.

activities implying reduced capacities for glycogen and glucose utilization. Enzyme activities in the phasic and tonic portions of the posterior adductor muscle of *M. squamosus* were virtually identical (Table I).

Metabolic responses to air exposure and recovery

Exposure to air for 12 h had no significant effect on the adenylate energy charge (Fig. 1) and the levels of arginine phosphate and ATP (Fig. 2) in the posterior adductor muscle of *G. demissa granosissima*. In contrast, adenylate energy charge and arginine

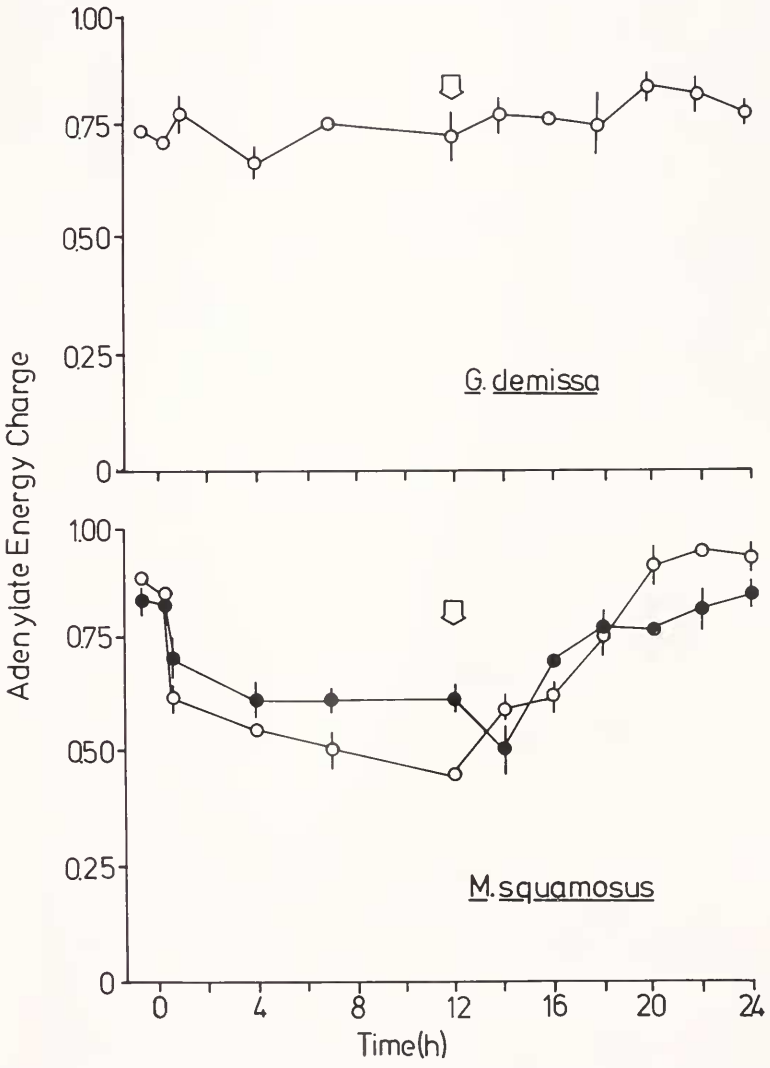


FIGURE 1. Alterations in the adenylate energy charge (ATP + ½ ADP ÷ ATP + ADP + AMP) in the posterior adductor muscles of *G. demissa granosissima* and *M. squamosus* during air exposure and recovery. Data for *M. squamosus* are given in terms of the phasic (solid circles) and tonic (open circles) adductor muscles. The initial time point is depicted slightly to the left of zero. The arrow indicates the onset of recovery. Each value is a mean ± 1 S.D. (n = 4).

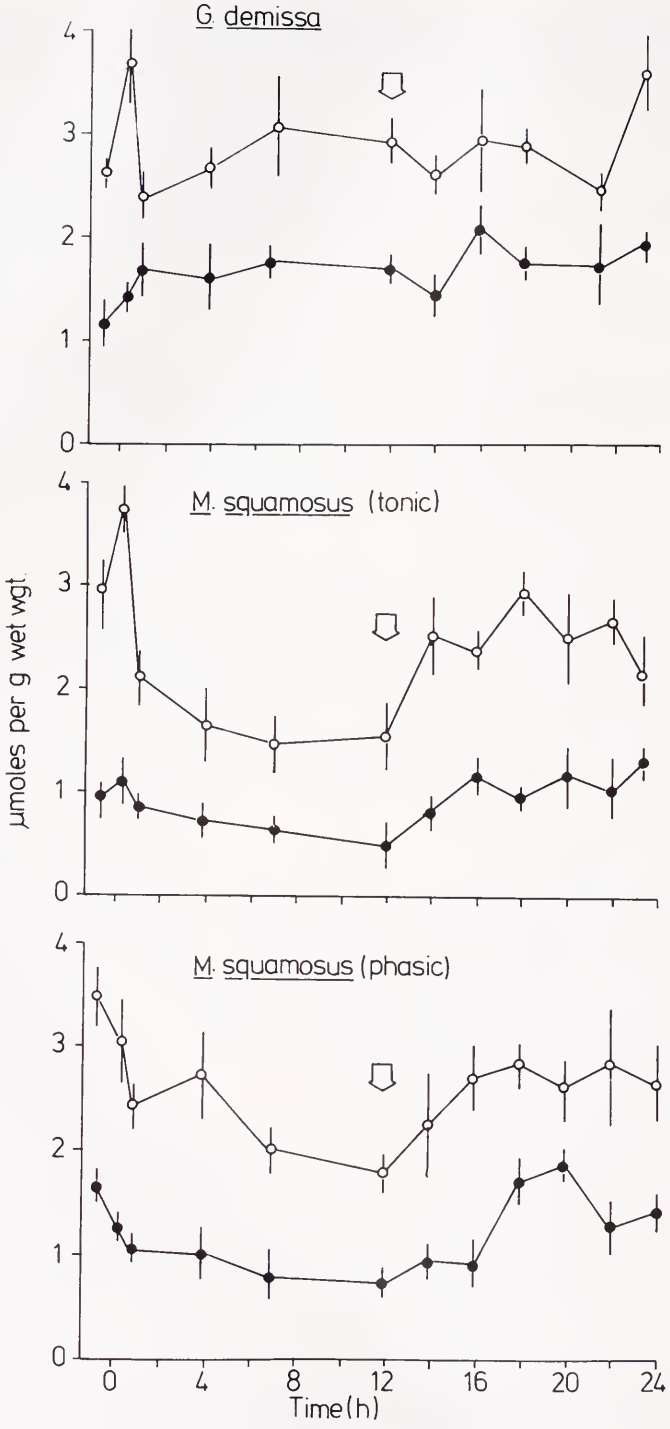


FIGURE 2. Effect of air exposure and recovery on the levels of arginine phosphate (open circles) and ATP (solid circles) in the posterior adductor muscles of *G. demissa granosissima* and *M. squamosus*. Each value is a mean ± 1 S.D. (n = 4).

phosphate and ATP levels fell significantly in both portions of the adductor muscle of *M. squamosus* (Figs. 1, 2). The greatest changes in these parameters occurred during the first two hours of air exposure. Changes in the high energy phosphates were most pronounced in the phasic adductor muscle of *M. squamosus*. During recovery after air exposure, there continued to be no changes in high energy phosphates in specimens of *G. demissa granosissima* (Figs. 1, 2). During recovery, the adenylates returned to initial levels in the posterior adductor muscle of *M. squamosus* (Figs. 1,

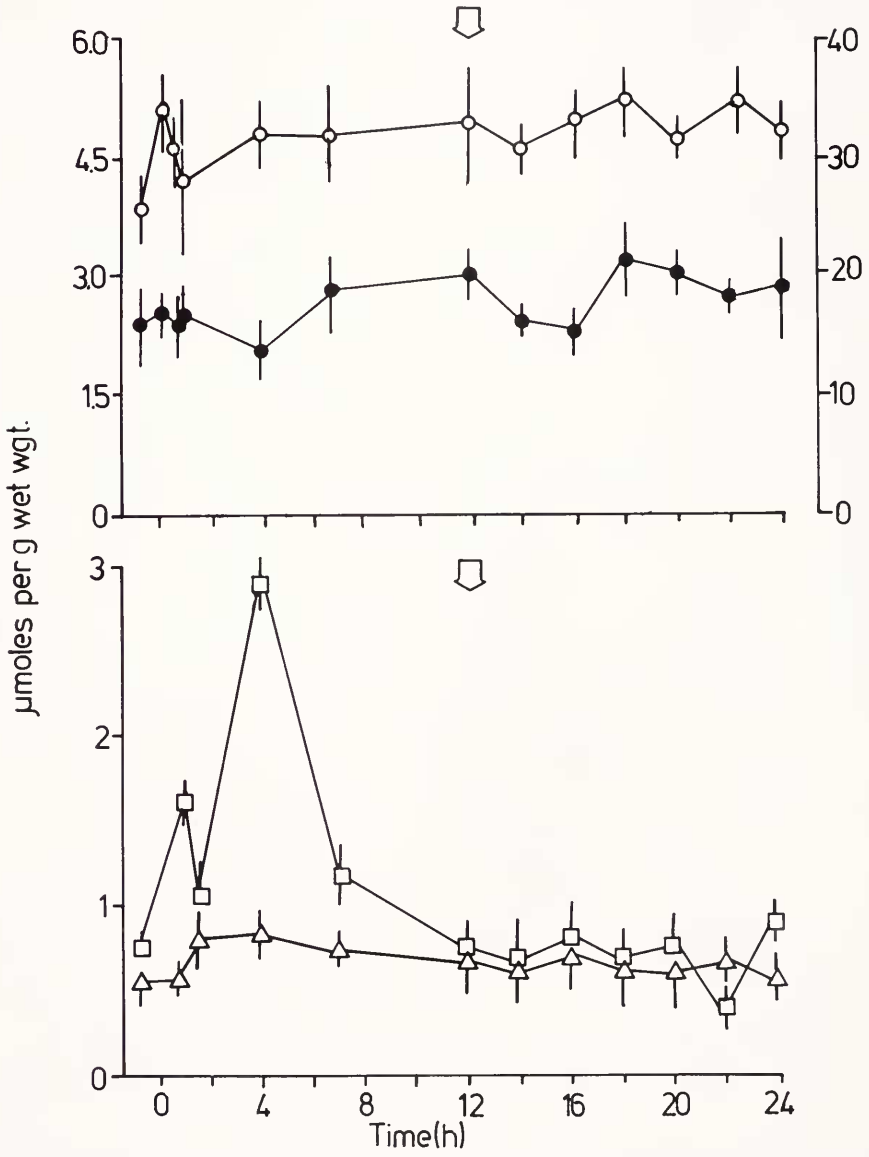


FIGURE 3. Effect of air exposure and recovery on the levels of alanine (open circles), aspartate (closed circles), succinate (squares), and D-lactate (triangles) in the posterior adductor muscle of *G. demissa granosissima*. Each value is a mean ± 1 S.D. (n = 4).

2). Arginine phosphate levels rose slowly during recovery but did not reach initial levels after 12 h of recovery (Fig. 2).

There were no significant changes in the levels of alanine, aspartate, and D-lactate

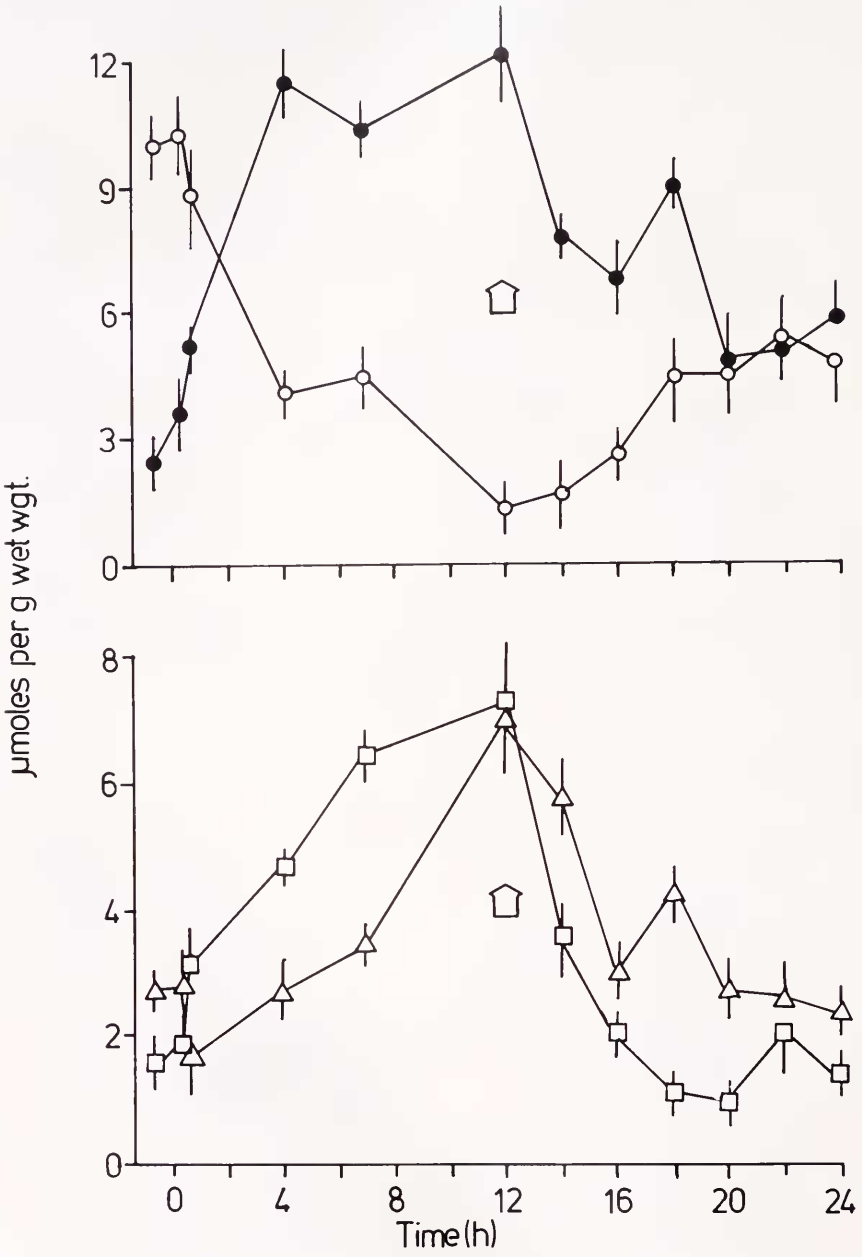


FIGURE 4. Effect of air exposure and recovery on the levels of alanine, aspartate, succinate, and D-lactate in the phasic adductor muscle of *M. squamosus*. Symbols are the same as in Figure 3. Each value is a mean ± 1 S.D. (n = 4).

during air exposure and recovery in the posterior adductor muscle of *G. demissa granosissima* (Fig. 3). There was a transient accumulation of succinate during the early period of air exposure, but succinate levels returned to the initial levels by the end of air exposure.

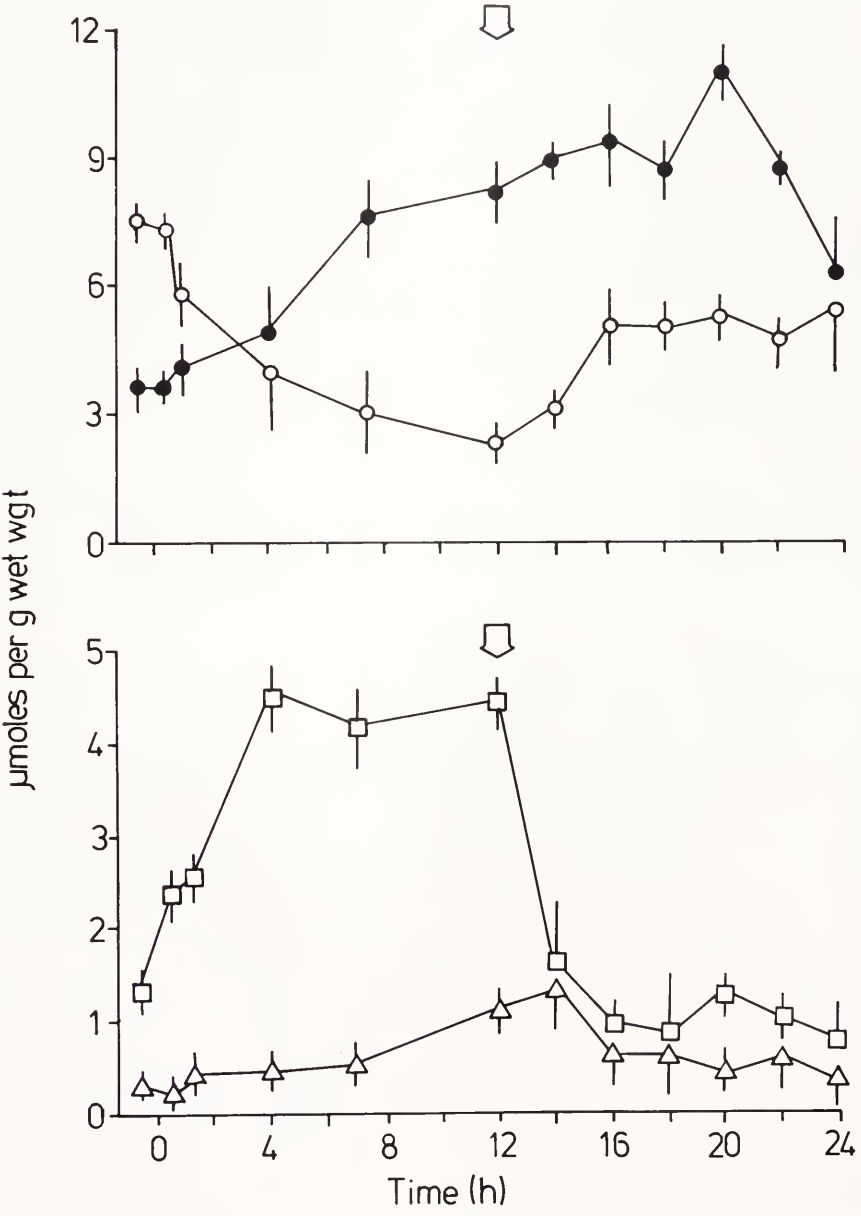
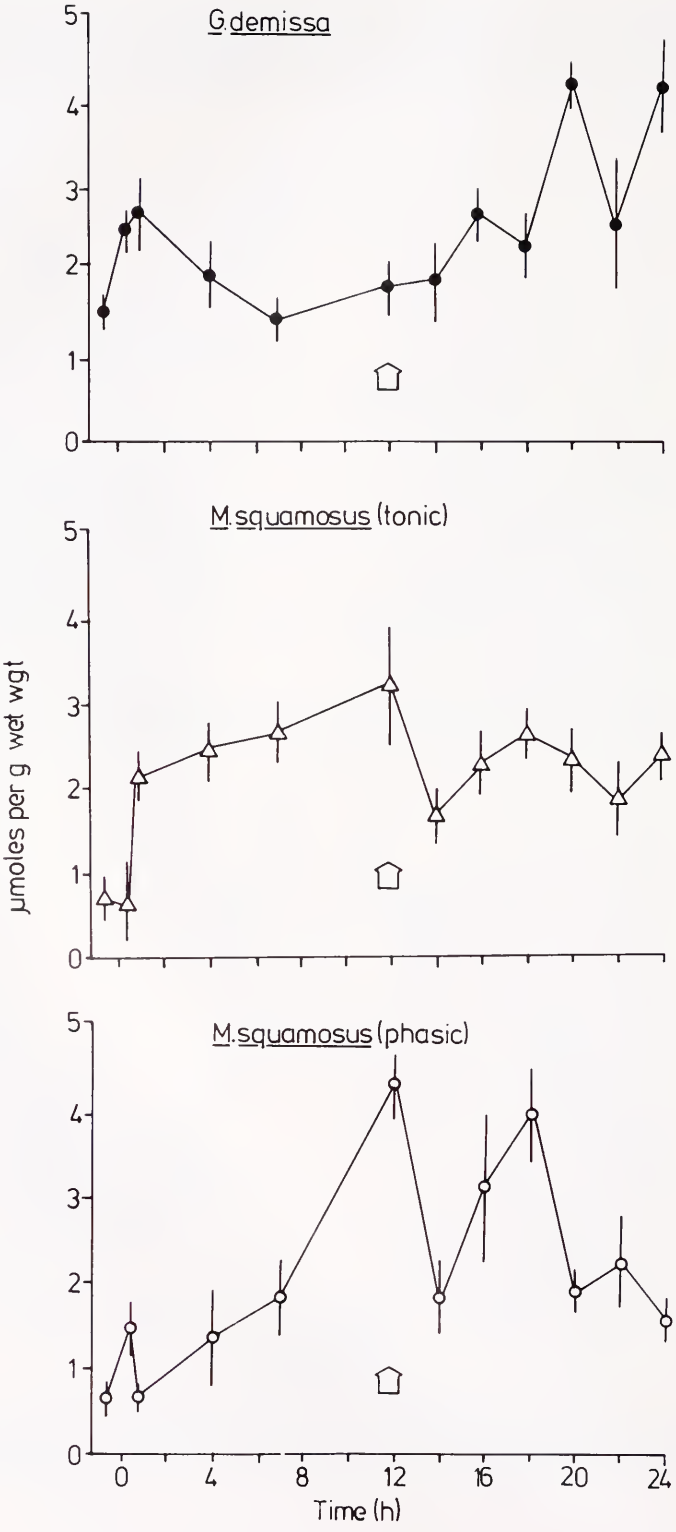


FIGURE 5. Effect of air exposure and recovery on the levels of alanine, aspartate, succinate, and D-lactate in the tonic adductor muscle of *M. squamosus*. Symbols are the same as in Figure 3. Each value is a mean ± 1 S.D. (n = 4).


G.demissa
M.squamosus (tonic)
M.squamosus (phasic)
μmoles per g wet wgt
Time (h)
0
1
2
3
4
5
0
4
8
12
16
20
24

There were pronounced changes in metabolite levels in the posterior adductor muscles of *M. squamosus*. In the phasic adductor muscle, aspartate levels declined throughout air exposure and there was nearly a stoichiometric increase in alanine levels (Fig. 4). There was a linear accumulation of succinate and D-lactate in the phasic adductor muscle (Fig. 4). A similar pattern of aspartate depletion and succinate and alanine accumulation was observed in the tonic adductor muscle of *M. squamosus* (Fig. 5). In contrast to the phasic adductor, the accumulation of D-lactate was low in the tonic adductor muscle during air exposure. The general patterns of recovery were similar in the phasic and tonic adductor muscle of *M. squamosus*. Succinate was rapidly cleared with initial levels being attained after 2–4 h of recovery (Figs. 4, 5). Aspartate levels increased during recovery and there was a gradual decline in alanine. After 12 h of recovery, alanine and aspartate levels still differed considerably from pre-air exposure levels. In the case of the phasic adductor muscle of *M. squamosus*, D-lactate levels slowly declined to initial levels during recovery (Fig. 4).

Alanopine/strombine accumulated during both air exposure and recovery in the adductor muscles of *G. demissa granosissima* and *M. squamosus* (Fig. 6). In the posterior adductor muscle of *G. demissa granosissima* there was an initial increase in alanopine/strombine during air exposure followed by a gradual decline. Alanopine/strombine levels then increased two-fold during recovery. In both the phasic and tonic adductor muscles of *M. squamosus*, alanopine/strombine accumulated throughout air exposure (Fig. 6). At the onset of recovery, there was a transient decline in alanopine/strombine followed by a period of further increase during the midpoint of the recovery period.

No significant changes in the levels of glycine and glutamate were observed in the adductor muscles of *G. demissa granosissima* and *M. squamosus*. In addition, there was no accumulation of octopine in either species. Propionate levels remained low during both air exposure and recovery.

Discussion

The results of this study show that there can be considerable intergeneric differences in terms of metabolic responses to air exposure in bivalve molluscs. The high intertidal mussel *Geukensia demissa granosissima* characteristically undergoes air gaping under these conditions. In contrast, the subtidal mussel *Modiolus squamosus* typically maintains tightly sealed valves during air exposure and displays air gaping only after extended periods of exposure. The consequences of these different responses to air exposure are strongly reflected in the patterns of energy metabolism in the tissues of these two species.

Air exposure for 12 h produced minimal metabolic stress on specimens of *G. demissa granosissima* as is evidenced by the lack of changes in high energy phosphates in the posterior adductor muscle. Although succinate and alanopine/strombine did accumulate during air exposure, the magnitude of the accumulation is small compared to that seen in other bivalve molluscs such as *Mytilus edulis* (de Zwaan *et al.*, 1983) and *M. squamosus* (this study). Thus, it appears that the anaerobic contribution to energy metabolism during the first 12 h of air exposure is minimal. Alanine and succinate accumulate to high levels in *G. demissa* after extended periods (>36 h) of incubation in oxygen free sea water (Ho and Zubkoff, 1982). Thus, this species has the capability of producing these end products under sufficiently stressful conditions.

FIGURE 6. Effect of air exposure and recovery on the levels of alanopine/strombine in the posterior adductor muscles of *G. demissa granosissima* and *M. squamosus*. Each value is a mean ± 1 S.D. (n = 4).

Specimens of *G. demissa* appear to be able to maintain significant rates of oxygen uptake during air exposure (Booth and Mangum, 1978). However, aerial oxygen consumption in this species is substantially less than aquatic oxygen consumption. Since rates of aerobic energy production are reduced during air exposure and there appears to be no large-scale utilization of anaerobic energy-producing pathways, the overall rates of ATP production in *G. demissa granosissima* posterior adductor muscle must fall during air exposure. Since the high energy phosphate levels are constant during air exposure, it is evident that overall rates of energy demand in the adductor muscle fall under these conditions. Thus, the apparent metabolic responses of the mussel *G. demissa granosissima* involve aerial gas exchange coupled with an overall reduction in the rates of ATP utilization in the posterior adductor muscle.

Air exposure produced dramatic alterations in the high energy phosphate levels in the phasic and tonic adductor muscles of *M. squamosus*. These alterations in high energy phosphates were similar in magnitude to what has been observed during anoxia in the tissues of a number of molluscs including the posterior adductor muscle of *M. edulis* (Ebberink *et al.*, 1979), the foot muscle of the cockle *Cardium tuberculatum* (Gäde, 1980), and the ventricle of the whelk *Busycon contrarium* (Ellington, 1981).

The simultaneous depletion of aspartate, and accumulation of succinate and alanine in both adductor muscles, indicates that glycogen and aspartate were fermented in *M. squamosus* during air exposure. This phenomenon has been consistently observed in a variety of molluscs (Collicutt and Hochachka, 1977; Ebberink *et al.*, 1979; Ellington, 1981). Collicutt and Hochachka (1977) predicted that both succinate and alanine accumulation should occur in a 1:1 ratio with aspartate depletion. However, all previous studies have shown that the amount of alanine accumulated was substantially greater than succinate. In the present study, the alanine:succinate accumulation ratio during the first 4 h of air exposure was 2.1 in the phasic adductor muscle of *M. squamosus*. However, in the tonic adductor muscle, the accumulation ratio was less than one during the first 4 h of air exposure and approached unity only after 12 h of exposure. Recently, de Zwaan *et al.* (1983) have explained accumulation ratios greater than one by suggesting that the mitochondrial malic enzyme is involved in shunting some aspartate-derived carbon in the direction of alanine synthesis. The rather different alanine:succinate accumulation ratios between the phasic and tonic adductor muscles of *M. squamosus* reflect variations in the metabolic disposition of malate derived from aspartate. There is also the possibility that some of the succinate production is derived from glycogen by the PEPCK route.

The accumulation of D-lactate in the phasic muscle and lack of accumulation in the tonic muscle is rather surprising in that the activities of LDH are virtually identical in the tissues. However, it must be noted that the decreases in high energy phosphates were much more pronounced in the phasic adductor muscle of *M. squamosus*. In addition, absolute levels of accumulation of alanine and succinate were much higher. Thus, rates of energy demand in the phasic muscle may be greater than the tonic muscle under these conditions. The lactate accumulation reflects increased glycolytic flux in this tissue. The simultaneous accumulation of lactate and succinate has also been observed in the foot muscle of the cockle *Cardium edule* (Gäde and Meinardus, 1981).

Recovery in the posterior adductor muscles of *M. squamosus* was characterized by the rapid clearance of succinate. Lactate was also cleared rapidly in the phasic muscle. Levels of ATP were rapidly restored. Arginine phosphate and aspartate slowly increased during the 12 h of recovery. Similar phenomena have been observed during recovery in the tissues of *M. edulis* (de Zwaan *et al.*, 1983) and *C. edule* (Gäde and Meinardus, 1981). In the present study, the time courses of succinate removal and

aspartate resynthesis were distinctly different indicating that there was probably no *direct* metabolic link during recovery between the two processes.

In specimens of both *M. squamosus* and *G. demissa granosissima* there was a transient production of alanopine/strombine during recovery from air exposure. Similarly, the bulk of strombine production in *M. edulis* occurred during recovery (Zurburg *et al.*, 1982; de Zwaan *et al.*, 1983). De Zwaan *et al.* (1983) found that the PO_2 levels in the hemolymph of the adductor muscle rapidly approached normoxic values during recovery. Thus strombine, a putative end product of anaerobic metabolism, was produced under essentially aerobic conditions. De Zwaan *et al.* (1983) rationalized this paradox by suggesting that energy demands exceed the limited capacity of aerobic ATP yielding processes in the tissue. Thus, there is an increase in glycolytic flux to meet the energy demands leading to strombine formation. The production of alanopine/strombine in the posterior adductor of *M. squamosus* during recovery can be easily interpreted by this argument. The production of alanopine/strombine in this species is coincident with the period of recharging of the adenylate pool. The post air exposure production of ananopine/strombine in *G. demissa granosissima* is more difficult to explain since there were no changes in high energy phosphates. However, increased energy demands might also result from other ATP requiring processes such as the possibility of increased contractile activity of the adductor muscle during recovery. It would be of great interest to measure valve movements during recovery in *G. demissa granosissima.*

The overall results of this study show that energy metabolism during 12 h of air exposure in the posterior adductor muscle of *G. demissa granosissima* is largely aerobic. Booth and Mangum (1978) suggested that the metabolism of the adductor muscle of *G. demissa* is largely anaerobic even in normoxic sea water. However, our results show that the role of anaerobic energy metabolism is minimal even under conditions of air exposure. This suggests that aerial gas exchange is sufficient to maintain adequate rates of ATP production. Furthermore, apparent reductions in energy demand tend to maintain energy balance in this tissue. In contrast, there are substantial decreases in high energy phosphates and an extensive reliance on anaerobic energy yielding processes during air exposure in the phasic and tonic adductor muscles of *M. squamosus.* These metabolic responses are probably due to a reduced capacity for aerial gas exchange, and, perhaps, smaller reductions in energy demands during air exposure. The patterns of aspartate and glycogen fermentation are similar to what has been observed in other molluscs. The differences in metabolic responses of *G. demissa granosissima* and *M. squamosus* to air exposure reflect differences in adaptation in micro-habitats of chronic *versus* infrequent air exposure.

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